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# SEÇÃO IV - FERTILIDADE DO SOLO E NUTRIÇÃO DE PLANTAS

# NITROGEN WASHING FROM $C_3$ AND $C_4$ COVER GRASSES RESIDUES BY RAIN<sup>(1)</sup>

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# **SUMMARY**

Crop species with the C<sub>4</sub> photosynthetic pathway are more efficient in assimilating N than C<sub>3</sub> plants, which results in different N amounts prone to be washed from its straw by rain water. Such differences may affect N recycling in agricultural systems where these species are grown as cover crops. In this experiment, phytomass production and N leaching from the straw of grasses with different photosynthetic pathways were studied in response to Napplication. Pearl millet (Pennisetum glaucum) and congo grass (Brachiaria ruziziensis) with the  $\mathrm{C}_4$ photosynthetic pathway, and black oat (Avena Strigosa) and triticale (X Triticosecale), with the C<sub>3</sub> photosynthetic pathway, were grown for 47 days. After determining dry matter yields and N and C contents, a 30 mm rainfall was simulated over 8 t ha-1 of dry matter of each plant residue and the leached amounts of ammonium and nitrate were determined. C4 grasses responded to higher fertilizer rates, whereas N contents in plant tissue were lower. The amount of N leached from  $C_4$  grass residues was lower, probably because the C/N ratio is higher and N is more tightly bound to organic compounds. When planning a crop rotation system it is important to take into account the difference in N release of different plant residues which may affect N nutrition of the subsequent crop.

Index terms: Cover crops, nitrogen fertilization, nutrient cycling.

**RESUMO**:  $LAVAGEM DO NITROGÊNIO DE RESÍDUOS DE PLANTAS <math>C_3$  E  $C_4$  PELA CHUVA

Espécies com via fotossintética  $C_4$  são mais eficientes na assimilação de N que plantas  $C_3$ , o que resulta em diferentes resistências do N do tecido à possibilidade de lavagem por

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chuva. Essa diferença pode alterar a ciclagem de N em sistemas agrícolas onde essas espécies são utilizadas como plantas de cobertura. O presente trabalho teve como objetivo estudar a produção de fitomassa em resposta ao N e à lavagem do nutriente da palha de gramíneas com diferentes vias de assimilação de carbono. Milheto (Pennisetum glaucum) e braquiária (Brachiaria ruziziensis), com via  $C_4$  e aveia-preta (Avena strigosa) e triticale (X. Triticosecale), com via  $C_3$  de assimilação de C, foram cultivados por 47 dias. Foi determinada a produção de matéria seca e tomadas amostras para determinação de N e C no tecido. Em seguida, foi simulada uma chuva de 30 mm, aplicada sobre o equivalente a 8 t ha $^{-1}$  de matéria seca de resíduos. Foram determinadas as quantidades de amônio e nitrato lavadas dos resíduos. As gramíneas  $C_4$  responderam a doses mais altas de fertilizante, mas os teores de N no tecido foram menores. A quantidade de N lavada dos resíduos foi menor para as plantas  $C_4$  provavelmente porque sua relação C/N foi maior e o N estava mais fortemente ligado aos compostos orgânicos da planta. No planejamento de uma rotação de culturas é importante ter em conta as diferenças na liberação de N dos resíduos, uma vez que isso pode alterar a nutrição nitrogenada da próxima cultura.

Termos de indexação: plantas de cobertura, adubação nitrogenada, ciclagem de nutrientes.

#### INTRODUCTION

One of the most important aspects to consider when choosing cover crop species is the N accumulation capacity, either by atmospheric N<sub>2</sub> fixation or uptake from soil, or both. However, N mineralization dynamics in cropping systems depend on factors such as the nature of organic N, C/N ratio of plant residues, N amount in the system and degree of contact of the tissues with soil colloids, as well as temperature, aeration, and moisture (Rosolem et al., 2004). The use of cover crops may result in significant increases not only in surface mulch and soil organic matter contents but also in nutrient cycling (Rosolem et al., 2006). Cultivated species of the Poaceae family (grasses) usually respond well to applied N. However, for grasses grown as cover crops the capacity to accumulate N is as essential as the speed at which N contained in plant residues is washed back to the soil with rains or mineralized after desiccation, which will determine when this N will be available for the following crop.

Some grasses have the C<sub>3</sub> photosynthetic pathway for C fixation, e.g. black oat (Avena strigosa), while others show the C<sub>4</sub> photosynthetic pathway, such as pearl millet (Pennisetum glaucum). This physiological discrimination influences N-release dynamics from the phytomass after the plants are cut (Rosolem et al., 2005). Tropical grasses e.g., as pearl millet, sorghum and Congo grass, have the  $C_4$  photosynthetic pathway and are more efficient in phytomass accumulation per unit of N taken up, compared with  $C_3$  plants such as wheat (*Triticum aestivum*) and black oat (Avena strigosa) (Taiz & Zeiger, 2002). Hence, after desiccation, the N-release dynamics from the phytomass of cover grasses may differ between  $C_3$ and C<sub>4</sub> species. As a result, N supply to the subsequent crop may vary in each crop rotation system, which should be taken into account in the plant residue management. The use of cover crops may result in significant increases not only in surface mulch and soil organic matter contents but also in nutrient cycling (Cantarella et al., 1997). One of the desirable traits in selecting cover crops species is the amount of N accumulated in the phytomass (Oliveira et al., 2002). Nevertheless, the proportion of N that will actually be used by the subsequent crop will depend on the synchronization between N release from the straw and the demand of the succeeding crop.

According to Taiz & Zeiger (2002), in  $C_4$ photosynthetic pathway plants, NO<sub>3</sub> is reduced and assimilated in leaf mesophyll cells, and CO<sub>2</sub> is incorporated in vascular bundle sheath cells, which increases the physiological efficiency in this plant group compared to C<sub>3</sub> plants, considering the use of light energy to assimilate N. Moreover, less than 10 % of the total N in C<sub>4</sub> plants leaves is used in the formation of the RuBP Carboxylase Oxygenase enzyme (Rubisco), and between 2 and 5 % is used in the formation of the PEP-carboxylase enzyme, whereas in C<sub>3</sub> plants Rubisco immobilizes from 20 to 30 % of the total N in the leaf tissue. The Rubisco enzyme accounts for approximately 50 % of the soluble proteins in the leaves of C<sub>3</sub> plants, compared with less than 10 % in C<sub>4</sub> plants. Therefore, there is greater N availability for the growth and development of plant tissues in C<sub>4</sub> plants, whereas in C<sub>3</sub> plants Rubisco works as a reserve of soluble N (Pimentel, 1998). Therefore, under equivalent environmental conditions. the shoots of C3 plants theoretically contain more available NH<sub>4</sub><sup>+</sup> per dry matter mass unit than C<sub>4</sub> plants (Foloni et al., 2009). Since N can be found in distinct forms in the leaf tissue of C<sub>3</sub> and C<sub>4</sub> plants, rain leaching of this nutrient from plant residues may be different in these groups of plants.

One hypothesis to explain the greater N-NH<sub>4</sub><sup>+</sup> leaching from plant residues in triticale and black oat (grasses) and in sunn hemp (legume) is the nature of N bounding to organic compounds resulting from

the  $C_3$  photosynthetic pathway for C assimilation (Rosolem et al., 2005). The intensity of N leaching from the straw of these species was higher than from  $C_4$  species under weak rainfall and tended to be similar to that of  $C_4$  plants, such as millet, guinea sorghum and congo grass under more intense rainfall. The amounts of N in  $C_3$  were higher than in  $C_4$  leaf tissue before rainfall. Thus, the higher the N content in the straw, the higher the amount of N washed away with rainwater, by a 70 mm rainfall (Rosolem et al., 2005).

If N assimilation and accumulation are different in  $C_3$  and  $C_4$  plants resulting in different N concentrations in the tissue and different dynamics in N release under rainfall, N cycling potential may be different for these species when grown as cover crops.

The objective of the experiment was to evaluate biomass production, N accumulation and N washed from residues of two  $C_4$  species (millet and congo grass) and two  $C_3$  species (black oat and triticale) by simulated rain, as related to N fertilization.

# MATERIAL AND METHODS

The experiment was carried out in a greenhouse in Botucatu, State of São Paulo, Brazil. The soil was collected from the arable layer (0–20 cm) of a distroferric Red Latosol (Embrapa, 1999) with 63 % sand, 4 % silt, and 33 % clay. The soil was air-dried and passed through a 2 mm mesh sieve. Chemical analyses (Raij et al., 2001) showed pH (CaCl<sub>2</sub>) 4.1, 17.0 g dm<sup>-3</sup> O.M., 3.0 mg dm<sup>-3</sup> P (resin), 69.0 mmol<sub>c</sub> dm<sup>-3</sup> H + Al, 0.3 mmol<sub>c</sub> dm<sup>-3</sup> K, 2.0 mmol<sub>c</sub> dm<sup>-3</sup> Ca, 1.0 mmol<sub>c</sub> dm<sup>-3</sup> Mg, and 4 % of base saturation. Soil water content at field capacity (-0.01 MPa) was 180 g kg<sup>-1</sup> (Embrapa, 1997).

Dolomitic lime (28 % CaO, 20 % MgO and ECC 95 %) was applied to increase base saturation up to 70 % (Raij et al., 1996), and the soil was moistened to field capacity and placed in plastic bags for 30 days. Then, 150 mg dm<sup>-3</sup> K (KCl) and 200 mg dm<sup>-3</sup> P (simple superphosphate) were applied. Subsequently, 14 dm<sup>3</sup> pots were filled with soil to reach the density of 1.2 g cm<sup>-3</sup>, so that similar field conditions would be represented. Nitrogen doses (0, 50, 100, and 200 mg dm<sup>-3</sup>) were applied as urea on soil surface and surface- incorporated (2 cm) into the soil at planting.

Seeds of pearl millet (*Pennisetum glaucum*, Linneu, cv. ADR-300), black oat (*Avena strigosa*, Schreb., cv. comum), congo grass (*Brachiaria ruziziensis*, Germain et Evrard) and triticale (*X. triticosecale*, Wittmack) were pre-germinated in the laboratory (using paper towels rolls) to minimize variations in the emergence time among species. After one week, 20 seeds of each species were planted per pot and, one week later, thinned to 12 plants per pot. Soil moisture was monitored daily by weighing the pots, and water was replaced as

needed. The pots were arranged in rows spaced 0.50 m apart, outside the greenhouse, for herbicide application, 47 days after planting. A non-selective systemic herbicide, Glyphosate, was applied at 1.0 kg ha<sup>-1</sup> (a.i.). The application was carried out with a CO<sub>2</sub> pressurized backpack sprayer with a spraying bar with four flat fan 110.04 nozzles (Spray Systems) spaced 0.40 m apart. Water volume and constant pressure for herbicide application were 40 mL m<sup>-2</sup> and 150 kPa, respectively. The bar was set 1.2 m above the soil surface and displacement speed was 1.0 m s<sup>-1</sup>. Then, the pots were brought back to the greenhouse.

Plant shoots were collected on the 7<sup>th</sup> day after herbicide application, by cutting at soil level. Plants were fragmented into pieces of 3–5 cm. One day before rain simulation, sub samples were taken, weighed (fresh), dehydrated in a forced air oven for 24 h at 60 °C, and weighed again (dry) to calculate water content. The amount of fresh straw to be used in the rainfall simulation (8.0 Mg ha<sup>-1</sup>) was calculated taking in account its water content. Part of the material was stored for N (sulfuric digestion and steam distillation) and C analysis (burning at 550 °C) as proposed by Embrapa (1997).

The rainfall simulator used was equipped with a bar supporting spraying nozzles, 1.45 m above the straw. Five high-flow nozzles (model TK-SS-20, Spray Systems) were used; nozzle spacing was 0.50 m. Traction in the spraying system was provided by belts and gears driven by electric motors, so that boom speed was maintained constant. The amount of rainfall applied at each boom passing was determined in previous tests, and the equipment was calibrated to apply a water volume of 2.5 mm. In other words, a 30 mm rainfall was simulated in 12 boom passings. The rainfall droplets produced by the simulator were generated at a pressure of 81 Pa, with a spray boom speed of 0.0925 m s<sup>-1</sup>.

The residues of the four species were placed within rigid PVC tubes (area 0.049 m<sup>2</sup>, height 0.10 m and bottom of 10 mm-mesh nylon screen). Each ring was filled with the equivalent to 8.0 Mg ha<sup>-1</sup> dry matter. The tubes were fixed on plastic funnels, and the water, after passing through the residues, was collected in containers placed under plastic curtains (to avoid contamination by water from simulated rain). The structures containing the plant residues were placed at equidistant points along the boom travel course. One ring without residues was also included in all rainfall simulations to estimate N contents in the water. At the end of each rainfall application, the water that had passed through the residues was collected and filtered. The volume was determined and the water cool-stored (2 °C). Later, the amounts of N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub> in the percolated water were determined by sequential distillation (Keeney & Nelson, 1982). After rainfall simulation, the straw was oven-dried at 60 °C for 72 h and N and C levels were determined.

The experiment design was a 4 x 4 factorial, arranged in completely randomized blocks, with four replications. Treatments consisted of four species grown under four N rates. The results were subjected to variance analysis. The t test  $(p \ge 0.05)$  was applied to compare different species in each N rate. Regression analysis was used to evaluate the effects of N fertilization for each species. Significant equations with the highest coefficients of determination (F test,  $(p \ge 0.05)$  were selected.

# RESULTS

The significance levels for shoot dry matter, N contents, N accumulation, C/N ratio, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> washed from the straw of different cover grasses as related to N fertilization were obtained by ANOVA (F test) (Table 1).

There was a linear increase in pearl millet dry matter yields with N fertilization and responses were observed in Congo grass up to a rate of 155 kg ha<sup>-1</sup>; both species are C<sub>4</sub>. The highest N rate tripled pearl millet dry matter yield and increased Congo grass phytomass 1.8 times. For triticale, a C<sub>3</sub> grass, a quadratic equation was fitted and the highest dry matter yield was observed with 137 mg dm<sup>-3</sup> N. Dry matter production of black oat was not increased with N rates (Figure 1).

Although pearl millet phytomass increased linearly with fertilizer rates (Figure 1), there was just a slight increase in tissue N concentrations at the highest N rate (Figure 2). The results, however, can be explained by a "dilution effect", since dry matter production of this crop was significantly increased (Figure 1). At the highest N rate the dry matter production of Congo grass was lower than of millet (Figure 1). Nevertheless, N concentrations in Congo grass plant tissue increased linearly with N fertilization up to 200 mg dm<sup>-3</sup> (Figure 2). Therefore, considering only the C<sub>4</sub> plants, pearl millet dry matter and N concentrations in Congo grass tissue increased with increasing N rates.

Black oat dry matter yields were not affected by N fertilization (Figure 1), but a quadratic response in plant tissue to N concentrations was observed (Figure 2), while N concentrations increased linearly in triticale.

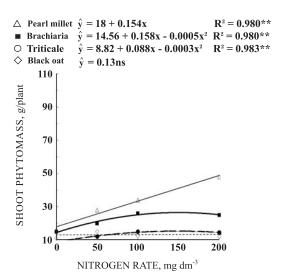
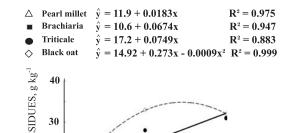


Figure 1. Shoot dry matter of cover grasses as related to N fertilization, 54 days after sowing. Vertical bar show the LSD comparing species ( $p \ge 0.05$ ). \*\*  $R^2$  significant (p  $\leq$  0.001); ns  $R^2$  non significant.



N CONTENT IN PLANT RESIDUES, g kg<sup>-1</sup> 20 50 100 150 200 NITROGEN RATE, mg dm-3

Figure 2. Nitrogen level in shoot dry matter of different cover grasses, as related to N fertilization, 54 days after sowing. Vertical bar show the LSD comparing species ( $p \ge 0.05$ ). \*\*  $R^2$  significant (p  $\leq$  0.001); ns  $R^2$  non significant.

Table 1. Significance levels (F test) for shoot dry matter, N contents, N accumulation, C/N ratio, NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub> washed from the straw of different cover grasses as related to N fertilization

Cause of variation	Dry matter	N content	N accumulation	C/N ratio	$\mathrm{NH_4}^+$	$NO_3$
Species	0.001	0.001	0.001	0.001	0.001	0.001
N rate	0.001	0.001	0.001	0.001	0.005	0.001
Species x rate	0.001	0.001	0.001	0.160	0.402	0.001

Under the simulated 30 mm rainfall, the amount of  $\mathrm{NH_4^+}$  washed from the  $\mathrm{C_3}$  plants, black oat and triticale was higher than from the  $\mathrm{C_4}$  species Congo grass and pearl millet (Figure 3a). The amount of  $\mathrm{NH_4^+}$  leached from triticale residues increased linearly with N rates, but  $\mathrm{NH_4^+}$  leaching from black oat straw increased only up to 152 mg dm<sup>-3</sup> N. Nevertheless, the highest amount of  $\mathrm{NH_4^+}$  was leached from black oat residues. In spite of a significant linear response for Congo grass, rain water washed very little  $\mathrm{NH_4^+}$  from pearl millet and Congo grass residues.

Black oat was the only crop with higher  $NO_3$  leaching at higher N rates (Figure 3b). At high N availability, N absorption may have been high enough to bring Nitrate Reductase activity to saturation, and eventually plant tissues accumulated nitrogen as free  $NO_3$ .

The total N accumulated in pearl millet, Congo grass and triticale plants increased linearly with N fertilization, whereas the response of N accumulated

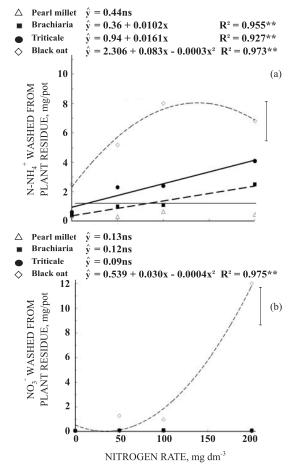


Figure 3. N-NH<sub>4</sub><sup>+</sup> (a) and NO<sub>3</sub><sup>-</sup> (b) leaching from cover crop plant residues after simulation of 30 mm rainfall as related to N fertilization. Vertical bar show LSD comparing species ( $p \ge 0.05$ ). \*\* R<sup>2</sup> significant ( $p \le 0.001$ ); ns R<sup>2</sup> non significant.

in black oat was quadratic (Figure 4). Hence, the tropical grasses Congo grass and millet were more efficient in extracting N from soil, although N levels in plant tissues were lower for these two crops than for black oat and triticale (Figure 2).

Pearl millet and Congo grass ( $C_4$ ), had higher C/N rates than black oat and triticale ( $C_3$ ), but N fertilization decreased the C/N ratios of all cover grasses (Figure 5).

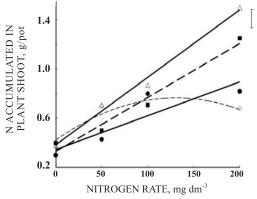


Figure 4. N accumulated in shoot dry matter of different cover grasses as related to N fertilization, 54 days after sowing. Vertical bar show LSD comparing species ( $p \ge 0.05$ ). \*\*  $R^2$  significant ( $p \le 0.001$ ); ns  $R^2$  non significant.

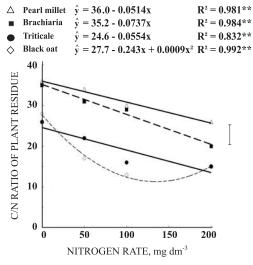


Figure 5. C/N ratio of the shoot dry matter of different cover grasses as related to N fertilization. Vertical bar show LSD comparing species ( $p \ge 0.05$ ). \*\*  $R^2$  significant ( $p \le 0.001$ ); ns  $R^2$  non significant.

# **DISCUSSION**

By modifying dry matter yields, N accumulation or the C/N ratio, N fertilization in plants with different photosynthetic pathways for C assimilation may affect N cycling and residue accumulation on the soil surface differently for the subsequent crop. Despite the fact that grasses are generally more N-demanding (Cantarella et al., 1997), tropical  $\mathrm{C}_4$  species such as sugar cane, maize, sorghum, congo grass, pearl millet, etc., are more efficient in using applied N for dry matter production and respond better to higher levels of applied N than temperate C<sub>3</sub> grasses, e.g., wheat, triticale, ryegrass, rye, black oat, etc (Lazenby, 1981). In general, increases in dry matter yields in the  $C_3$ species were lower in our experiment (Figure 1), but N concentrations in plant tissues were higher than in Congo grass and millet, in response to applied N. According to Pimentel (1998), in C<sub>3</sub> species such as black oat and triticale, N demand is high for the formation of the Rubisco enzyme, which accounts for about 50 % of the total amount of soluble proteins in leaves, generally resulting in higher N levels in leaf tissues and lower C/N ratios in plant residues. On the other hand,  $C_4$  species are more efficient in  $CO_2$ fixation and consequently, N is highly available for the formation of new tissues. Therefore, N levels usually decrease in the phytomass of these species, which is known as "dilution effect" (Pimentel, 1998). In this study, lower N concentrations were found in Congo grass and millet residues, with a slight increase in leaf N, probably due to a higher amount of straw produced per unit of N applied (Figures 1 and 2).

Rosolem et al. (2005) reported  $NH_4^+$  leaching from six cover crops (black oat, millet, triticale, sunn hemp, Congo grass, and sorghum) varying from 2.5 to 9.5 kg ha<sup>-1</sup>, depending on rainfall, but without biological decomposition of the residues. The authors classified the species into two distinct groups:  $C_3$  plants (triticale, black oat and Indian hemp), with higher  $NH_4^+$  leaching; and  $C_4$  grasses with lower  $NH_4^+$  leaching. Findings by Rosolem et al. (2005) revealed that N was more readily leached by rain water from  $C_3$  than from  $C_4$  plant species per unit of phytomass. Results of the current study support the earlier findings of Rosolem et al. (2005). Plant tissue N concentrations were higher in  $C_3$  (black oat and triticale) than in  $C_4$  grasses (Congo grass and millet), regardless of the N fertilizer rates (Figure 2).

Physiological efficiency of  $C_4$  species is higher than of  $C_3$  plants, considering the low energy use to assimilate N in the former. These crops use less N to form enzymatic complexes for  $CO_2$  assimilation, and therefore, there is more N available for the growth and development of plant tissues in  $C_4$  plants (Marschner 1995; Pimentel 1998; Taiz & Zeiger 2002). This explains the higher response of Congo grass and millet to N fertilization (Figure 1). On the other hand, in the  $C_3$  species black oat and triticale large amounts

of N were possibly consumed in the formation of the Rubisco enzyme and the capacity of C assimilation was also lower, resulting in reduced growth and eventually in lower N uptake. Hence,  $C_4$  crops were more efficient than  $C_3$  plants in N uptake and use.

Usually, plant residues can be classified in two groups; fast and slow decomposition types, i.e., residues with C/N ratios higher than 25 strongly immobilize N and have a low decomposition rate; and on the contrary, C/N ratios below 25 indicate high N mineralization rate and fast plant residue decomposition (Boer et al., 2007). Generally, the higher the N rate, the lower the C/N ratio observed in the plant residues, which decreased from 36 to 23, 35 to 19, 24 to 11 and 28 to 10 for millet, congo grass, triticale and black oat, respectively. Despite the high capacity of C<sub>4</sub> grasses of accumulating N in phytomass (Figure 4), the results of this experiment suggest that tropical species have a lower decay rate and immobilize greater amounts of N during decomposition, compared with temperate  $C_3$  crops.

Values of C/N ratio were inversely proportional to N levels (Figures 2 and 5). This indicates a C/N ratio decrease due to higher N levels in plant shoots as a result of higher N fertilization. In terms of plant residue management and crop rotation systems. Congo grass and pearl millet showed higher increases in phytomass and N accumulation at the highest N rate of 200 mg dm<sup>-3</sup> (Figures 1 and 4). However, N recycling to the soil in a system with these tropical C<sub>4</sub> grasses may be delayed due to high C/N ratios (Figure 5); this could affect the nutrition of the subsequent crop grown in no till systems (Aita & Giacomini, 2003). Results for black oat and triticale differed, i.e., the highest N dose (200 mg dm<sup>-3</sup>) for triticale and the calculated dose of 140 mg dm<sup>-3</sup> of N for black oat increased N content and decreased C/N ratios of the straw (Figures 2 and 5). In an experiment using litter bags, N release from Amaranthus cruentus (C<sub>3</sub>) was faster than than from Pennisetum glaucum and *Eleusine coracana* (C<sub>4</sub>), which confirms our results (Boer et al., 2007). Therefore, in systems where these C<sub>3</sub> grasses are grown, at least part of the fertilizer N to be applied to the cash crop could be applied to the cover crops, which would recycle the N to the soil faster than C<sub>4</sub> species. This could be an important strategy of preventing N losses from agricultural

When choosing cover crop species preceding the main crop it is important to consider the low N recycling observed since the N release from cover crop residues should be synchronized with the subsequent crop demand. In some cases,  $C_3$  grasses may be preferred in view of their ability to release N leaching at faster rates to the soil (Figure 3a). When cover crops are grown aiming at high amounts of phytomass,  $C_4$  species should be chosen, as they produce higher straw yield, irrespective of N fertilization (Figure 1).

# CONCLUSIONS

- 1.  $C_4$  species accumulate more dry matter and nitrogen than  $C_3$  species, but the nutrient is less washed by rains.
- 2. Under conditions where N losses and cycling are important, C<sub>3</sub> species might be a better option.

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